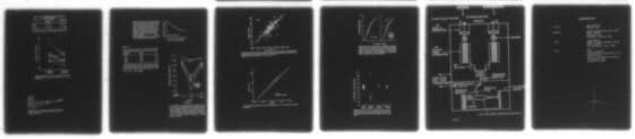
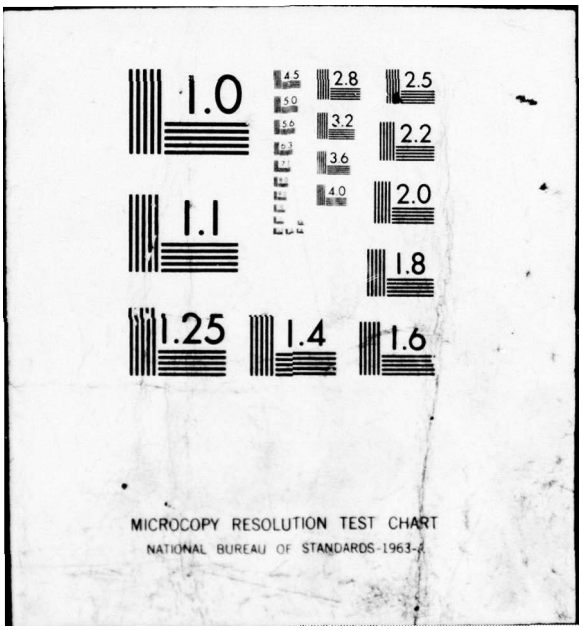
AD-A045 207 ROCHESTER UNIV N Y SCHOOL OF MEDICINE AND DENTISTRY F/G 6/5
STUDIES ON METABOLISM, FUNCTION AND MECHANISM OF DESTRUCTION OF--ETC(U)
NOV 76 M A LICHTMAN, J COHEN, A DANIEL DADA17-73-C-3135

UNCLASSIFIED NL

| OF |
AD
A045 207



END
DATE
FILMED
11-77
DDC


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

AD A045207

DDC FILE COPY

AU NO.

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER	Progress
4. TITLE (and Subtitle)	5. TYPE OF REPORT & PERIOD COVERED
Studies of Metabolism, Function and Mechanism of Destruction of Red Cells.	Annual Report, 1 Sep [illegible] 31 Oct 1976
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s)	8. CONTRACT OR GRANT NUMBER(s)
Marshall A. Lichtman, M.D. Jules Cohen, M.D. Alan Daniel, M.D.	DADA17-73-C-3135
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
University of Rochester School of Medicine and Dentistry Rochester, New York 14642	62772A 3S762772A814 00.072
11. CONTROLLING OFFICE NAME AND ADDRESS	12. REPORT DATE
US Army Medical Research and Development Command Washington, DC 20314	November 1976
	13. NUMBER OF PAGES 16p.
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report) Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

DDC RECEIVED OCT 13 1977 B

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

2,3 Diphosphoglycerate acid

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

hemoglobin, 2,3-DPG, red cell ATP, erythrocytes, oxygen consumption, blood flow, anemia, blood rheology, hemoglobin oxygen affinity

The authors, P sub 50, oxygen tension

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

We have developed a simplified method for measuring oxygen hemgolobin affinity. This method takes advantage of the observation that the Po_2 of peripheral venous blood is in effect tonometered by the tissues to a value around the P_{50} of hemoglobin. By using an equation into which the antecubital venous pH, Po_2 and So_2 is inserted, one can calculate the P_{50} of the patients' hemoglobin. This has proved to be a useful (cont on p 1473 B)

oxygen tension and oxygen saturation

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

307300

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

(cont fr P1473 A)

technique for the identification of mutant hemoglobins with high or low affinity.

Using a dog gracilis muscle model for the study of oxygen transport, we have determined that at normal flow rates, severe alkalosis does not impair either resting or exercising oxygen consumption in spite of the associated reduction in P_{50} in vivo. During alkalosis, however, the muscle functions at a lower level of venous Po_2. Thus, at a reduced arterial oxygen flow rate, the induced affinity change may have an impact on tissue oxygen uptake. This is under study.

+ two

P sub 50

oxygen tension.

STUDIES OF METABOLISM, FUNCTION AND MECHANISM OF DESTRUCTION OF RED CELLS

ANNUAL PROGRESS REPORT

September 1, 1975 to October 31, 1976

Marshall A. Lichtman, M.D.
Jules Cohen, M.D.
Alan Daniel, M.D.

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D.C. 20315

Contract No. DADA17-73-C-3135

University of Rochester School of Medicine and Dentistry
Rochester, New York 14642

Progress Report for Contract No. DA17-73-C-3135 November 1, 1975 to October 31, 1976

1. Effect of hemodialysis on intraerythrocytic phosphate compounds and oxygen binding to hemoglobin.

We have studied the effects of shifts in pH and plasma inorganic phosphate P_i on subjects undergoing hemodialysis. Plasma inorganic phosphate concentration fell significantly during the period of dialysis, although red cell inorganic phosphate was not influenced by this reduction (Table 1). The uptake of inorganic phosphate by the red cell was shown to be relatively rapid. Intravenous infusion of inorganic phosphate salts to a subject with chronic hypophosphatemia resulted in a proportional and prompt increase in red cell P_i. Red cell 2,3-DPG and ATP did not change significantly in six hours.

Exodus of inorganic phosphate from the red cell is probably very slow. This was confirmed by our studies. Normal human red cells were placed in a phosphate-free, but otherwise physiologic, salt solution containing 0.5 g/100 ml of human albumin for six hours. Intracellular P_i did not fall during this period of observation.

Red Cell 2,3-DPG content was not altered by six hours of hemodialysis. Red cell ATP was also not changed by hemodialysis. Since the distribution ratio of plasma to red cell inorganic phosphate concentration expressed as μmoles/ml H_2O did not fall below unity during dialysis and the exodus of inorganic phosphate is slow, red cell inorganic phosphate content is not threatened by dialytic therapy as currently performed.

Plasma pH was increased during six hours of hemodialysis. Blood base changed from a slight average deficit to a moderate average excess. The net effect of the rise in blood pH and development of base excess was a slight increase in oxygen binding by hemoglobin (Figure 1). The increase in oxygen binding was modest as indicated by a mean decrement in P_{50} *in vivo* of 0.9 torr. The increase in oxygen binding by hemoglobin was not associated with postdialysis symptoms. Two subjects had symptoms of headache and/or nausea at the termination of dialysis. In one, P_{50} *in vivo* decreased by 1.0 torr; in another, P_{50} *in vivo* increased by 0.5 torr. Four subjects with the largest decrease in P_{50} *in vivo* did not have late or postdialysis symptoms (Figure 2).

2. Red cell adenosine triphosphate in hypoproliferative anemia with and without chronic renal disease: Relationship to hemoglobin deficit and plasma inorganic phosphate.

We have found that red cell ATP content, known to be elevated in subjects with anemia of chronic renal disease, was elevated, also, in the red cells of subjects with hypoproliferative anemia without renal disease. In anemic subjects, with and without renal disease, the increase in red cell ATP was associated with the extent of hemoglobin deficit; however, the increment in red cell ATP was greater in subjects with chronic renal disease at a given reduction in hemoglobin concentration. In subjects with chronic renal disease, red cell ATP content was also strongly correlated with plasma inorganic phosphate (P_i) concentration. The latter relationship appeared to explain the additional increase in red cell ATP, although these studies do not allow conclusions as to causality. Normal and

reduced plasma P_i concentrations were associated with a reduced red cell ATP content for a given hemoglobin deficit in subjects with chronic renal disease. Red cell magnesium was elevated in subjects with chronic renal disease and in subjects with hypoproliferative anemia. Reticulocyte ATP was three-fold the mean population ATP concentration in normal subjects. This difference fits an exponential decay in red cell ATP with aging. It is possible therefore, that age-dependent hemolysis may explain population red cell ATP content in hypoproliferative anemias. Selective changes in the age of red cell populations may explain the quantitative variation in mean red cell ATP levels (Figure 3).

3. Effects of contrast materials on red cell membrane potential and plasma and red cell pH.

In these studies, contrast materials have been shown to produce an acute reduction in blood pH. By adding impenetrable organic anions to the external milieu, the electrical effect of internal red cell organic anions is counteracted and the negative potential across the red cell membrane is nullified. The red cell membrane potential (E) expressed as volts may be calculated from the equilibrium distribution of chloride by use of the Nernst equation:

$$E = -\left[\left(\frac{RT}{zF}\right) \ln \frac{[Cl^-]_e}{[Cl^-]_i}\right]$$

where R, the gas constant, equals 8.314 joule/°K per mole; T, the abolute temperature, is 310°K; z, the valence of the chloride ion, is unity; F, the Faraday constant, is 96493 coulomb/eq; and $[Cl^-]$ is the concentration of chloride in plasma water (e) and in cell water (i). The activity coefficient of Cl^- is assumed to be the same in cytosol as in plasma.

The anion equivalency of impenetrable compounds in the red cell is approximately 70 mEq/L H_2O. This total is composed of hemoglobin ($\sim$ 40 mEq/L), 2,3-diphosphoglycerate ($\sim$ 20 mEq/L), adenosine triphosphate ($\sim$ 5 mEq/L) and other phosphates ($\sim$ 5 mEq/L). If the effect of contrast material is due to its balancing, the internal impenetrable anions, the membrane potential should be obliterated when approxomately 70 mEq/L H_2O of sodium Hypaque is added to the external milieu.

In order to test this hypothesis, we prepared isotonic sodium Hypaque solutions and added isotonic sodium chloride so as to achieve solutions of 0 to 135 mM sodium Hypaque which were isotonic (280-300 mOsm). Since 1 mM sodium Hypaque produced 2 mOsm, we assumed complete dissociation of the compound in solution. We, thereafter, titrated the equivalents of extracellular Hypaque anion required to reduce the membrane potential to zero and beyond, as shown in Table 5. Addition of Hypaque resulted in a reduction and finally inversion of the membrane potential as increasing concentrations of Hypaque were added. By extrapolation, the membrane potential was zero at $\sim$ 65 mEq/L of Hypaque which is very close to the expected average value of 70 mEq/L (Table 2). This reduction in negativity of the inside with respect to the outside of the membrane allows the rate of influx of permeable anions, hydroxyl, bicarbonate and chloride to increase and equal or exceed that of efflux. The greater buffering capacity of the interior of the red cell and the increase in Pco_2 in the

red cell prevent a significant change in internal pH, whereas plasma is acidified by the net increase in hydrogen ion concentration which occurs.

Coronary sinus blood was sampled following injection of Renografin or Hypaque into the coronary artery. pH measured in blood collected 5 to 8 seconds after dye injection decreased in each of four dogs studied (Figure 4). Red cell pH did not change significantly (not shown). The fall in pH was closely associated with the concentration of dye present in the blood samples studied. The magnitude of the pH fall observed was greater than that in renal blood, due to the ability to sample more rapidly. If 0.5 M $NaHCO_3$ was added to Hypaque solutions prior to injection, the fall in plasma pH was prevented, although in this case red cell pH increased significantly (e.g., 7.19 to 7.25). The abrupt change in plasma pH could contribute to the abnormality of membrane and cell function, which may occur following injection of contrast material, especially into the coronary circulation.

4. The use of a single venous blood sample to measure the in vivo P_{50} of hemoglobin.

The affinity of hemoglobin for oxygen is of clinical importance because it may decrease in response to hypoxia, anemia or reduced blood flow and acts, thereby, to maintain venous (i.e. tissue) PO_2 as oxygen extraction increases. In situations in which the oxygen-hemoglobin equilibrium is altered as a consequence of deficits in oxygen content or flow, the effect of the four major determinants of the equilibrium, that is red cell, 2,3-DPG, pH, temperature, and Pco_2 must be considered. This has been called the oxygen-hemoglobin dissociaton curve at in vivo conditions and is represented by the P_{50} in vivo (P_{50} iv).

An assessment of the affinity of hemoglobin for oxygen is considered inaccessible to the practicing physician and hematologist since it requires tonometry and mixing techniques often *available only in research* laboratories. In the following studies, we examined the usefulness of a single venous blood sample as an indicator of the position of the oxygen-hemoglobin dissociation curve. A single venous blood sample, analyzed for pH, PO_2 and SO_2 by a clinical laboratory could be used by a physician to assess the presence of an alteration in the oxygen-hemoglobin equilibrium.

Venous blood samples were obtained from healthy subjects as well as those with hypoproliferative anemia, congestive heart failure, ischemic heart disease, acidosis, alkalosis, sickle cell disease and from umbilical cord blood.

The CALCULATED (CALC) P_{50} iv of venous blood at 37°C was determined from the formula:

$$\text{CALC } P_{50}\text{iv} = \text{antilog } (\log P_{50}\ \text{std} + 0.48\ (7.40\text{-pH}) + 0.0013\ (\text{BE}))$$

where P_{50} std was derived for a hemoglobin-oxygen dissociation curve.

The AS RECEIVED (AS REC) P_{50} iv was determined from the formula:

$$\text{AS REC } P_{50} \text{ iv} = \text{antilog}\left[\frac{\log 1/k}{n}\right]$$

where

$$1/k = \text{antilog } (n \log PO_2) \; X\left[\frac{100-SO_2}{SO_2}\right]$$

PO_2 and SO_2 were measured in the venous blood. The Hill Constant, n, was considered to be 2.7 in all subjects. If necessary, AS REC P_{50} iv can be adjusted for body temperature by the formula:

$$P_{50} \text{ iv} = \text{antilog } [\log P_{50} + 0.024 \; (t-37°C)]$$

where t is subject's body temperature at the time of sampling. All formulae were entered into a Wang 600 programmable calculator.

Figure 5 depicts the relationship in 102 subjects between AS REC P_{50} iv at 37°C determined by a single PO_2 and SO_2 measured in venous blood and the CALC P_{50} iv at 37°C determined by, firstly, preparing a three to five point oxygen-hemoglobin dissociation curve, secondly, deriving the P_{50} std from the curve and, thirdly, adjusting the P_{50} std to the pH and base excess of the venous blood at the time of sampling. A strong and highly signifcant correlation was present ($r = 0.8$, $P < 0.001$). Moreover, the slope of the curve was such that AS REC P_{50} iv was in excellent agreement with CALC P_{50} iv above a P_{50} of 26 torr and was only slightly greater (maximally 0.5 torr) than CALC P_{50} iv as P_{50} decrease from 26 to 21 torr.

Eleven subjects with either alkalosis or acidosis were studied also. CALC P_{50} iv derived from the P_{50} std, pH and base excess or deficit was very similar to the AS REC P_{50} iv based on a single venous blood sample. The correlation coefficient for the association of CALC P_{50} iv with AS REC P_{50} iv determined from a single venous blood sample was very high ($r = 0.9$, $P < 0.001$).

An important question regarding the AS REC P_{50} iv is the validity of using venous rather than arterial blood to assess P_{50} iv. Since P_{50} std is the same in venous and arterial blood, the major concern is the difference in pH of venous as compared to arterial blood. We, as others, have found that arterial pH is very closely correlated with venous pH. Figure 6 depicts the relationship of central venous to arterial pH based on 170 observations from arterial and venous blood samples measured simultaneously. A very high correlation ($r = 0.93$, $P < 0.001$) was present. Mean venous pH was about 0.03 pH units less than arterial at an arterial pH of 7.40.

In situations where tissue lactate production is very high, as in shock, use of venous blood to measure P_{50} *in vivo* may be misleading since red cell pH would not be influenced by blood lactate during the time required for capillary transit. Since CO_2 rapidly enters the red cell and is hydrated instantaneously by the action of red cell carbonic anhydrase, increases in Pco_2 as blood traverses tissue capillaries can produce changes in oxygen-binding to hemoglobin that may be functionally important. Under all but the most extreme circumstances the pH gradient between artery and vein is related to differences in Pco_2. Indeed, in attempting to quantify the effect role of oxygen binding to hemoglobin on oxygen delivery the P_{50} gradient from artery to vein may be the most valid estimate, excluding the effect of excess lactate. Central rather than peripheral venous blood is most useful for measuring P_{50} *in vivo*.

5. The use of a single venous blood sample to measure P_{50} at standard conditions so as to detect mutant hemoglobins.

Also, measuring the strength of oxygen binding to hemoglobin at standard *in vitro* conditions is of clinical importance because it is a means of detecting the presence of a mutant hemoglobin with high or low affinity for oxygen. Hemoglobins with markedly altered affinity for oxygen may be the cause of polycythemia or, less commonly, anemia.

The evaluation of patients with polycythemia is hampered by the requirement for special equipment to do oxygen-hemoglobin dissociation curves. These instruments, found in a few laboratories, are often inaccessible to the practicing physician. We show here that measurement of venous blood, pH, oxygen tension (Po_2), and oxygen saturation (So_2), as performed in a clinical chemistry laboratory, is a useful means for detecting hemoglobin with an altered affinity for oxygen.

$$\text{AS REC } P_{50} \text{ std} = \text{antilog } \frac{\log 1/k}{n},$$

$$\text{where } 1/k = [\text{antilog } (n \log Po_{2(7.4)})] \cdot \frac{100-So_2}{So_2}$$

A Hill constant (n) for hemoglobin A of 2.7 was used in all calculations. The Po_2 in venous blood, measured at 37°C was converted to PO_2 at pH 7.4 with the formula:

$$\log Po_{2(7.4)} = \log Po_2 - [0.5\ (7.40-pH)],$$

where pH represents the value in the antecubital venous blood.

The $Po_{2(7.4)}$ and So_2 of antecubital venous blood from 38 healthy subjects are shown in Figure 7A. These observations fall near the curve expected for the oxygen-hemoglobin equilibrium of normal blood. The shaded area in Figure 7B depicts the range for healthy subjects. The $Po_{2(7.4)}$ and So_2 of antecubital venous blood from healthy subjects with polycythemia vera and hypoxic polycythemia fall within the range for healthy subjects. The $Po_{2(7.4)}$ and So_2 of the antecubital venous blood from subjects with structural variants of hemoglobin with altered oxygen affinity fall outside the range for healthy subjects.

The distribution of P_{50} calculated from the venous $Po_{2(7.4)}$ and So_2 is shown in Figure 8. The blood of healthy subjects had a mean P_{50} std of 26 ± 1.3 (S.D.) mm Hg. The 99% confidence interval for individual observations was 22.6 to 29.4 mm Hg. The P_{50}'s derived from six observations in three subjects with hemoglobin Bethesda and a single observation in subjects with either hemoglobin's Olympia, Rainier, or Yakima, all high-affinity hemoglobins, were outside the 99% lower confidence limit for healthy subjects. Also, two observations in one subject with >90% hemoglobin S, who was known to have erythrocytes with low-oxygen affinity from prior studies of her oxygen-hemoglobin dissociation curve, were far outside the upper confidence limits for healthy subjects.

The AS REC P_{50} std of subjects with hemoglobin Bethesda (∿ 15 mm Hg) was somewhat higher than the P_{50} std measured with a full oxygen-hemoglobin dissociation curve (∿ 9 mm Hg). This was explained by the profound deviation of the oxygen-hemoglobin dissociation curve of this mutant hemoglobin from the sigmoid curve that is described mathematically by the Hill equation. Nevertheless, the P_{50} std was markedly abnormal and detected the oxygen binding abnormality unequivocally. In subjects with hemoglobin's Olympia, Rainier, Yakima, and S, the AS RECEIVED P_{50} std was within 1 mm Hg of the P_{50} obtained from a full oxygen hemoglobin dissociation curve. In previous studies, a close correlation of AS RECEIVED P_{50} std with P_{50} std derived from a full curve in subjects with normal hemoglobin was found.

6. The role of hemoglobin-oxygen affinity in oxygen transport to ischemic myocardium.

Fourteen patients, ten with angina and four with atypical chest pain were studied during diagnostic cardiac catherization and coronary angiography. An attempt was made to re-examine the suggestion that a decrease in hemoglobin-oxygen binding occurs across the coronary bed during pacing induced angina. It has been further suggested that this decrease is not explained by any of the known determinants of P_{50}. We were not able to confirm these observations that had been reported from another laboratory. Utilizing careful paired sample analyses, we could not detect any changes during pacing-across the coronary circulation--in P_{50} measured at standard conditions (pH 7.4, Pco_2 40 torr, T 37°C), or in red cell 2,3-DPG level. What we did find was that patients developed respiratory alkalosis during pacing. This alkalosis was sufficient to produce a significant reduction in P_{50} at in vivo conditions of pH. There was an average fall in P_{50} of 1.6 torr, and a decrease of 3.0 torr was observed in one patient. Thus, a significant increase in hemoglobin-oxygen binding was observed. Patients with coronary disease, and those with normal coronaries all became alkalotic. The mechanism appeared to be a hyperventilatory response to pacing since a consistent fall in P_ACO_2 accompanied the alkalosis. If hyperventilation and respiratory alkalosis occur during spontaneous angina, then in regions of the coronary vascular bed where flow is fixed, the fall in P_{50} might be deleterious to oxygen release and compound the limitation in oxygen transport caused by restricted blood flow.

7. The role of hemoglobin-oxygen affinity in oxygen transport during congestive heart failure.

Twenty two patients with varying degrees of chronic cardiac decompensation were studied during diagnostic cardiac catherization. The interrelationships among arterial oxygen flow rate (OFI_A), oxygen binding by hemoglobin and whole body oxygen utilization were examined. Despite a reduction of 63% in systemic oxygen transport from the highest to the lowest OFI_A, oxygen consumption was relatively well maintained because there was an increase in proportional extraction of oxygen, in close association with falling OFI_A. There was also an increase in P_{50}, both at standard and at in vivo conditions as OFI_A fell, and rising proportional extraction and P_{50} were significantly correlated with one another (r=0.50). We calculate that about one-third of the increase in proportional extraction of oxygen that was observed, as OFI_A fell, could be explained by rising P_{50}, that is--by a decrease in hemoglobin affinity for oxygen in tissue capillaries. Thus, altered hemoglobin oxygen affinity appears to be an important adaptive mechanism for maintaining tissue oxygen utilization when systemic oxygen transport is impaired in patients with chronic heart disease.

8. Gracilis muscle model for studies of oxygen transport.

We have developed an isolated muscle model to test the hypothesis that altered hemoglobin-oxygen binding can influence tissue oxygen uptake when blood flow and arterial blood oxygen content are held constant (Figure 9). The model is a variation of that described by Renkin in Acta Physiol. Scand. 54: 223, 1962.

In our initial experiments, the dog gracilis muscles were isolated, the gracilis artery and vein were cannulated and the muscle was perfused with blood that had been collected earlier the same day from the same dog. The blood had been treated in one of several ways to modify Hb-O_2 affinity, then oxygenated and passed through a finger pump into the muscle. Various blood treatment modalities were tested, including (1) blood storage in ACD to reduce 2,3-DPG levels, (2) exposure to metabisulfite (3) treatment with potassium cyanate to carbamylate the hemoglobin. All such treatments appeared to produce red cell damage and perhaps sludging so that muscle vascular resistance rose dramatically during blood infusion, and interpretation of the data was difficult. These manipulations will, nevertheless be pursued, as these findings may have an important bearing on oxygen transport when patients are transfused with stored blood.

At present, however, we are autoinfusing the muscle from the donor dog and are manipulating hemoglobin-oxygen affinity by inducing respiratory alkalosis (Bohr effect). In a typical experiment, the following protocol is followed after muscle isolation and establishment of controlled flow:

1. Control gracilis arterial (A) and venous (V) blood sampling
2. Induction of respiratory alkalosis by hyperventilation
3. Repeat A, V sampling for determination of resting muscle $\dot{V}O_2$ and lactate production
4. Stimulation of the muscle for approximately one minute and measurement of $\dot{V}O_2$ and lactate production during exercise
5. Collection of blood samples during recovery period
6. Restoration of ventilation to normal is followed by a period to allow recovery of muscle to basal conditions
7. Repeat steps 3-5

8. Alteration of blood flow rate to the muscle and repeat of steps 3-7

9. At each step blood samples are taken for determination of blood pH, PO_2, Pco_2, $\%HbO_2$, %HbCO, Hb level, lactate concentration. Such sampling allows not only calculation of $\dot{V}O_2$ and lactate production but of P_{50} at both standard and *in vivo* conditions

10. At each step, the muscle is also subjected to a test for arterial occlusion to be sure that it has retained its capacity to autoregulate and is thus behaving physiologically.

The preliminary data suggests that in the range of normal flow rates, alkalosis does not impair either resting or exercise $\dot{V}O_2$ in spite of the associated reduction in P_{50} *in vivo*. However, during alkalosis, the muscle appears to operate at lower levels of venous PO_2. It is possible, then that with further reduction in arterial flow rate, or with more extreme exercise, induced affinity changes may have an impact on tissue O_2 uptake.

Articles published, in press or submitted for publication with support of Contract DA17-73-C-3135 in 1975 and 1976:

1. Lichtman, M.A. The effect of hemodialysis on intraerythrocytic phosphate compounds and oxygen binding to hemoglobin. Kidney International Suppl. 2:S-134, 1975.

2. Lichtman, M.A. and Murphy, M.S. Red cell adenosine triphosphate in hypoproliferative anemia with and without chronic renal disease: Relatinship to hemoglobin deficit and plasma inorganic phosphate (Proceedings of a conference on Stress Erythropoiesis, May, 1975, Rochester, New York) Blood Cells 1: 467, 1975.

3. Lichtman, M.A. Does ATP decrease exponentially with red cell aging? Nouv. Rev. Franc. D'Hematol. 15: 625, 1975.

4. Lichtman, M.A. and Lipchik, E.O. Acidification of the plasma by the red cell in the presence of radiographic contrast materials: a possible cause of adverse effects on the heart. Trans. Assoc. Amer. Phys. 88: 265, 1975.

5. Lichtman, M.A., Whitbeck, A.A. and Murphy, M.S. Factitious changes in binding of oxygen to hemoglobin when based on extracellular pH in the presence of certain blood additives like radiographic contrast media. Invest. Radiol. 10: 225, 1975.

6. Lichtman, M.A., Murphy, M.S., Whitbeck, A.A., Pogal, M., and Lipchik, E.O. Acidification of plasma by the red cell due to radiographic contrast materials. Circulation 52: 943, 1975.

7. Lichtman, M.A. and Murphy, M.S. Reduced red cell membrane potential and acidification of the plasma in response to contrast materials: Time course of the alteration in plasma pH. Invest. Radiol. Accepted for publication.

8. Lichtman, M.A., Murphy, M.S., and Pogal, M. The use of a single venous blood sample to assess oxygen binding to hemoglobin. Brit. J. Haemat. 32: 89, 1976.

9. Lichtman, M.A., Murphy, M.S., and Adamson, J.W. A simplified technique for the detection of mutant hemoglobins with altered affinity for oxygen. Ann. Int. Med. 84: 517, 1976.

10. Daniel, A., Cohen, J., Lichtman, M.A. Changes in chemoglobin-oxygen binding during atrial tachypacing in subjects with anginal chest pain. Amer. J. Cardiol. Submitted for publication.

11. Daniel, A., Lichtman, M.A., Cohen, J. The relationship between arterial oxygen flow rate, oxygen binding to hemoglobin and oxygen utilization in patients with cardiac failure. In preparation.

Table 1. Effect of hemodialysis on red cell and plasma inorganic phosphorus

	Predialysis	Postdialysis
Plasma P_i	2.01 ± 0.32	1.14 ± 0.19
Red cell P_i	0.58 ± 0.093	0.57 ± 0.098

Values represent the mean ± SEM of μmoles/ml of plasma or cells.

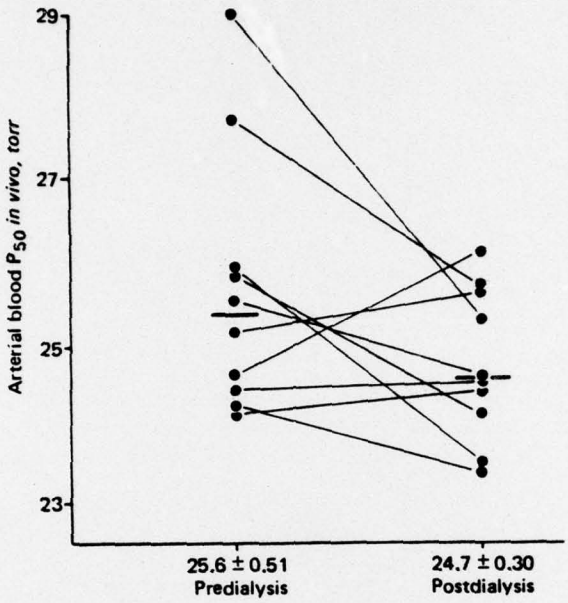


Fig. 1 *Arterial blood P_{50}* in vivo *before and after hemodialysis*. The horizontal bar represents the median value. The mean ± SEM is also shown below.

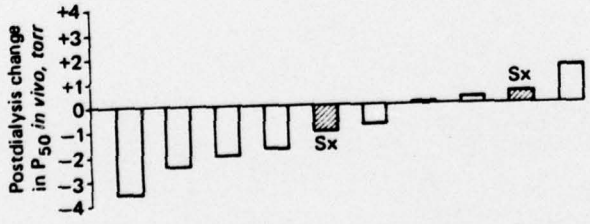


Fig. 2 *The change in P_{50}* in vivo *after six hours of dialysis is shown for ten subjects studied*. Two subjects had late dialysis symptoms (Sx) of headache and/or nausea.

Fig. 3 The mean red cell ATP content of red cell populations according to the population life-span. The arrows indicate the mean red cell ATP for anemic subjects with and without chronic renal disease. If age-dependent shortening of survival explains the elevated red cell ATP content, the survival of red cells would be 50 and 80 days for those with and without chronic renal disease respectively. The variation in red cell ATP in subjects with anemia 4) would, in part, be explained by the relative contributions of age-dependent hemolysis in each subject

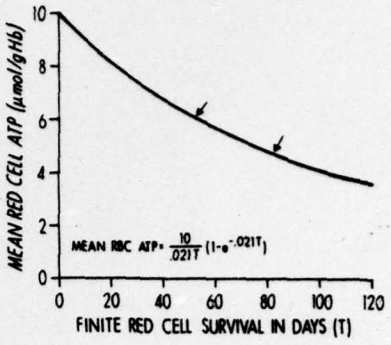


Table 2

Red Cell Membrane Potential (E) in the Presence of Hypaque

Hypaque concentration (M)	$\ln \frac{[Cl^-]_e}{[Cl^-]_i}$	E (mV)
0.0	+0.48	−12.8
0.045	+0.14	− 3.7
0.075	−0.07	+ 1.9
0.090	−0.30	+ 8.0
0.135	− 1.1	+29.4

Final concentration in solution was 0.150 M in each case. NaCl concentration was equal to 0.150 M minus the Hypaque concentration. E represents potential of the inside of red cell membrane with respect to the outside expressed in millivolts. $E\ (mV) = -26.7 \ln \frac{[Cl^-]_e}{[Cl^-]_i}$

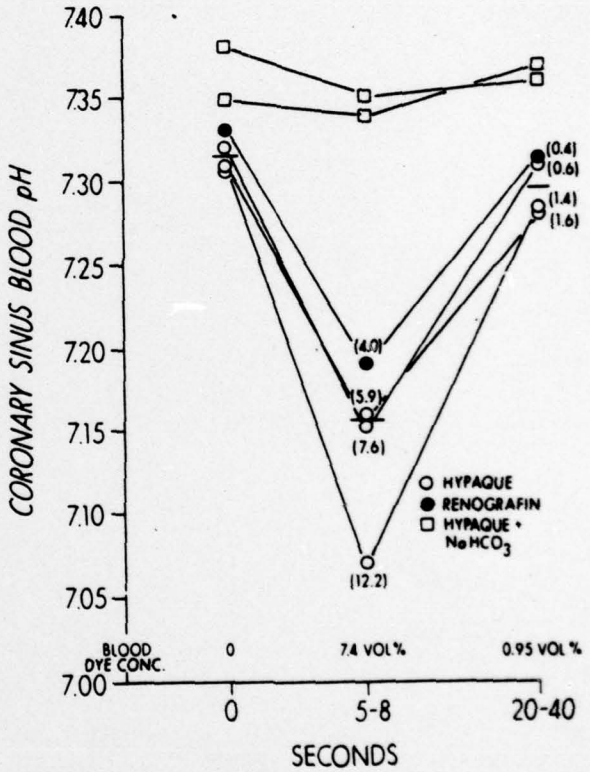


Figure 4

The extracellular pH in dog coronary sinus blood before, 5 to 8 seconds, and 20 to 40 seconds after injection of Hypaque (unfilled circles) or Renografin (filled circles). The mean blood contrast material concentration is shown in ml contrast/100 ml blood (vol %). The unfilled squares represent the blood pH when Hypaque to which 0.5 M $NaHCO_3$ was added was injected into the coronary artery. The parentheses contain the concentration of contrast material in the blood.

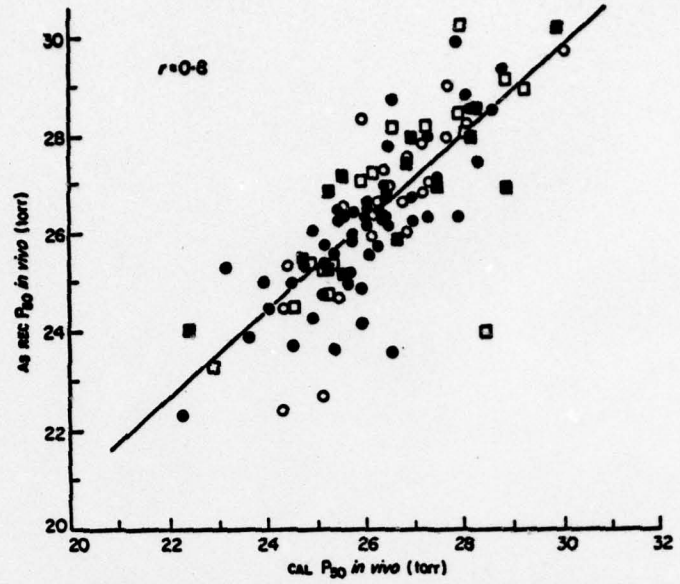


FIG 5 The association of AS REC P_{50}iv with CALC P_{50}iv in healthy subjects (○) and subjects with hypoproliferative (reticulocytopenic) anaemia (□), ischaemic heart disease (●) and congestive heart failure (■) is shown. AS REC P_{50}iv = 0.91 (CALC P_{50}iv) + 2.60.

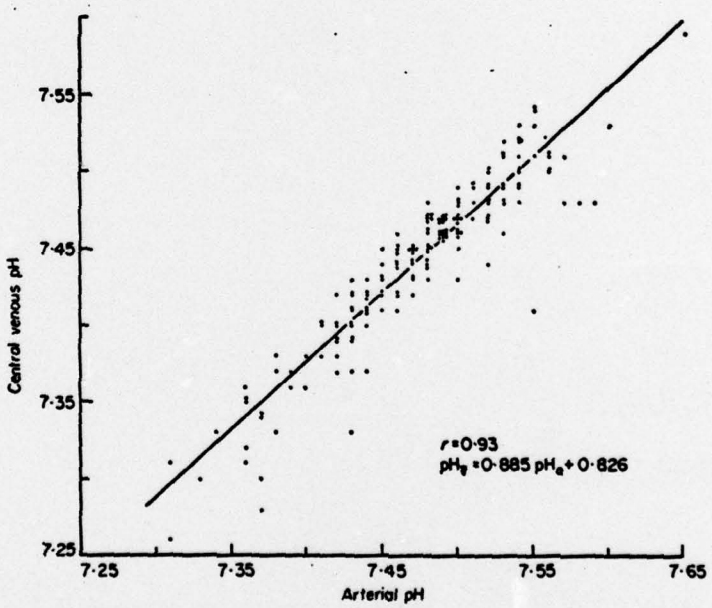


FIG 6 The relationship of central venous pH to arterial pH is depicted. 170 observations in 62 subjects are included.

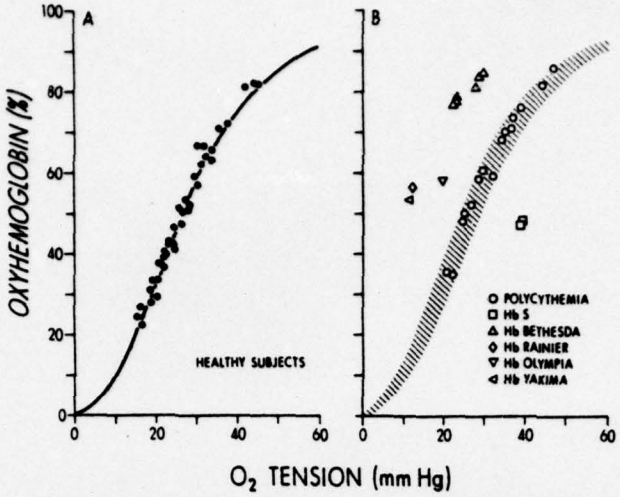


Figure 7A. *The percent of hemoglobin as oxyhemoglobin at the antecubital venous Po_2, corrected to pH 7.4, is shown for 38 healthy subjects. B. The shaded area depicts the normal range for healthy subjects. The circles represent observations in 5 subjects with hypoxic polycythemia and 10 with polycythemia vera. The triangles, squares, and diamonds represent observations in subjects with mutant hemoglobins with altered oxygen affinity.*

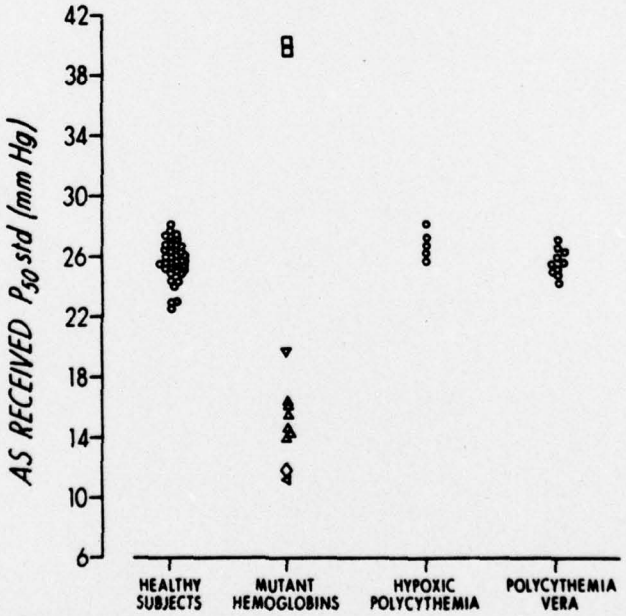


Figure 8. The AS REC P_{50} std's calculated from the antecubital venous blood pH, Po_2, and So_2 are shown for healthy and polycythemic subjects and those with mutant hemoglobins. The symbols for subjects with mutant hemoglobins correspond to those in Figure 7B.

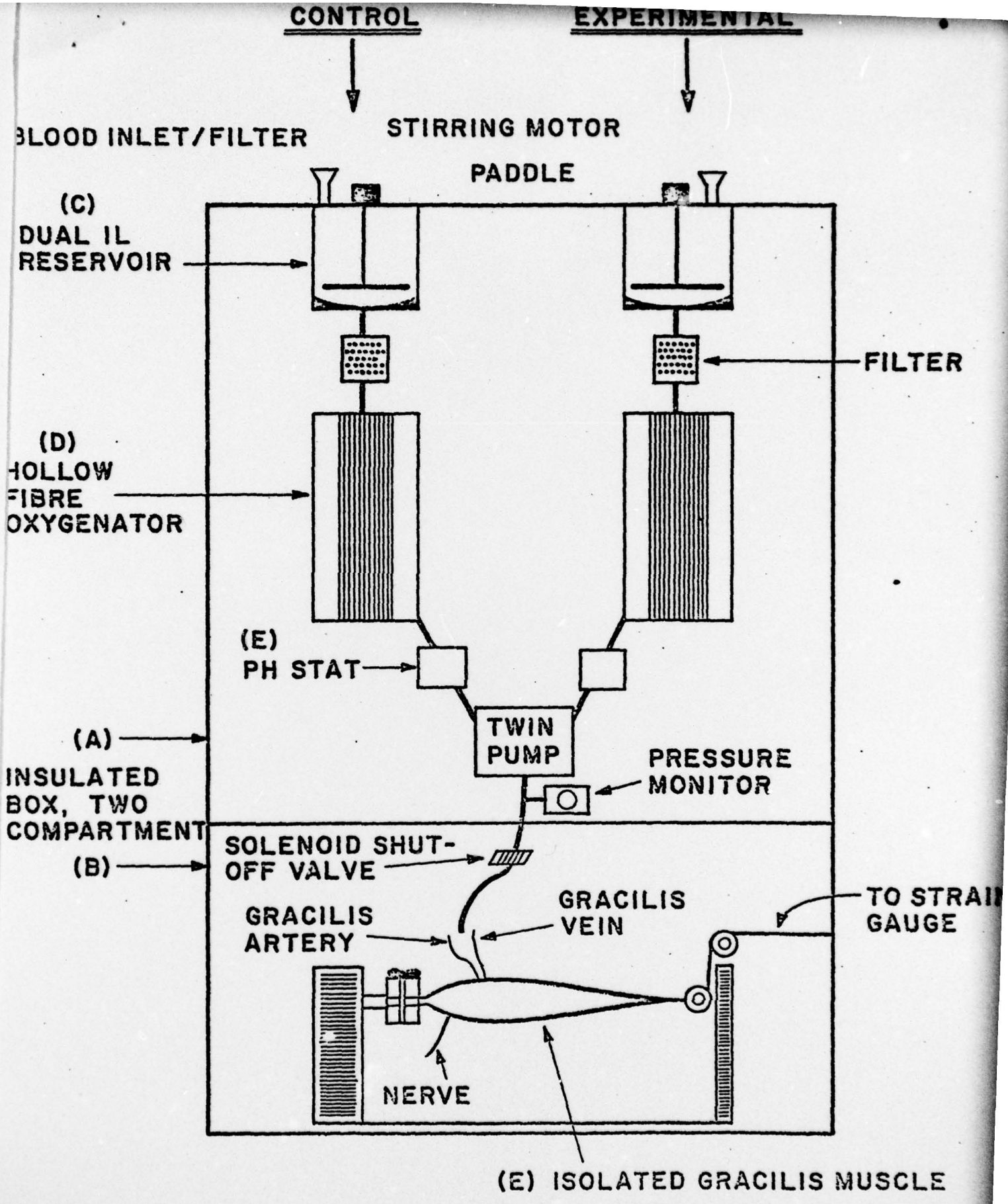

CONTROL
EXPERIMENTAL
BLOOD INLET/FILTER
STIRRING MOTOR
PADDLE
(C)
DUAL IL
RESERVOIR
FILTER
(D)
HOLLOW
FIBRE
OXYGENATOR
(E)
PH STAT
TWIN
PUMP
PRESSURE
MONITOR
(A)
INSULATED
BOX, TWO
COMPARTMENT
(B)
SOLENOID SHUT-
OFF VALVE
GRACILIS
ARTERY
GRACILIS
VEIN
TO STRAIN
GAUGE
NERVE
(E) ISOLATED GRACILIS MUSCLE

Figure 9

DISTRIBUTION LIST

4 copies	HQDA (SGRD-RP) WASH DC 20314
12 copies	Defense Documentation Center (DDC) ATTN: DDC-TCA Cameron Station Alexandria, Virginia 22314
1 copy	Superintendent Academy of Health Sciences, US Army ATTN: AHS-COM Fort Sam Houston, Texas 78234
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences Office of the Secretary of Defense 6917 Arlington Road Bethesda, MD 20014